Free Radical Scavenging Activity of *Chromolaena odorata* L. Leaves

Putri Anni Maulida, Devi Anggraini Putri, and Sri Fatmawati

***Abstract*—*Chromolaena odorata* is one of the plants used by the community as traditional medicine. Some of community in Ambon, Indonesia used *C. odorata* leaves as wound medicine. This study aims to evaluate free radical scavenging activity from fraction of methanol extract of *C. odorata*. Methanol extract of *C. odorata* leaves is known have good free radical scavenging activity. The fractionation from methanol extract of the *C. odorata* leaves obtained A-E fraction, where fractions C, D and E showed inhibitory activity against DPPH radicals (2,2-difenill-1-pikrilhidrazil) with IC_{50} values 63,95; 64.38 and 202.15 µg/mL. Positive control, which is gallic acid, has an IC_{50} value of 5.29 µg/mL.**

Keywords—***Chromolaena odorata*, Antioxidant, DPPH, IC_{50}.**

I. Introduction

Chromolaena odorata known as kirinyu is a weed which is widely distributed in Indonesia. *C. odorata* is one of the flowering shrub plants in the *Asteraceae* family. *C. odorata* can be found in subtropical and tropical regions, such as Asia, Africa, Australia and South America [1]. *C. odorata* estimated to have spread in Indonesia since 1910 [2] mainly in Lombok, Sumba, Sumbawa, Flores, Timor, Rote, Alor, Sulawesi, and Papua [3].

C. odorata is a shrub plant with a height of 1.5-2 m and a maximum height of 6 m [4]. This plant has a soft stem with a base of woody stems. Flowers from *C. odorata* are white to pink with an amount of about 10 to 35 flowers (Figure 1). The leaves are triangular to elliptical with jagged edges. The leaves are 4-10 cm long and 1-5 cm wide, and the base of the leaves is 1-4 cm in size. The seeds of the plant *C. odorata* are rather hairy and can be dispersed by the wind and stick to clothing and machinery because of their long-range distribution [5].

C. odorata has been used as a traditional medicine in several countries including Indonesia. People in Ambon Indonesia used leaves of *C. odorata* for wound healing [4]. *C. odorata* is also used in Nigeria and Vietnam as wound healing and antiinflammatory [6][7]. Water extract from *C. odorata* leaves is used as a medicine for diarrhea, malaria and diabetes [8]. *C. odorata* reports has bioactivity as antidiabetic [9][10], antioxidants [1][11], antimicrobials [12], anticataract [10], antihelmatics and wound healing [14].

Phan reported several secondary metabolites in *C. odorata* leaf extract, such as alkaloids, saponins, tannins, flavonols (tamarixetin and kaemferida), flavonoons (eupatilin), chalcones and phenolic acids such as ferulic acid and protocatric acid [13]. Methanol extract from the leaves of *C. odorata* is also reported contain metabolites secondary, including: alkaloids, tannins, phlobatanin, steroids, terpenoids, flavonoids and cardiac glycosides [14].

C. odorata have been used as traditional medicine. However, reports of free radical scavenging activity of *C. odorata* have not been widely reported. This study focuses on the evaluation free radical scavenging activity from the fraction of methanol extract *C. odorata* leaves.

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TABLE 1.
ANTIOXIDANT ACTIVITY WITH DPPH RADICAL INHIBITION AND IC_{50} VALUE FROM A-E FRACTION

Sample	Inhibition (%) ± SD^n	IC_{50} (µg/mL)
Fraction A	24,61 ± 0,26	>322,55
Fraction B	36,24 ± 2,52	>322,55
Fraction C	81,41 ± 0,27	63,95
Fraction D	81,44 ± 0,68	64,38
Fraction E	52,34 ± 3,13	202,15
Gallic acid	94,19 ± 0.42	5,29

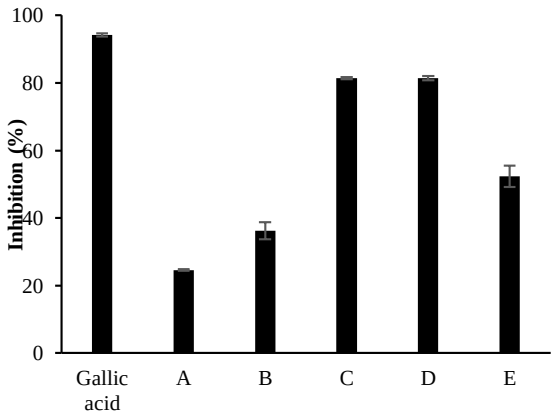

Figure 1. The inhibitory activity of the positive control is gallic acid, and the sample (A-E fraction) to DPPH radical (concentration of each sample 322.55 µg / mL). Each sample shows an average value of ± SD, n = 3.

II. Material and Method

A. Chemicals

The chemicals used were 2,2-diphenyl-1-picryl-hydrazyl (DPPH) (TCI, 1898-66-4), methanol (Merck), gallic acid. Solvents (*n*-hexane, dichloromethane, ethyl acetate and methanol).

B. General Experimental Procedures

The compounds was determined by coloumn chromatography (CC) using silica gel 60 G (Merck). The absorbance data measured by UV-Vis Genesys Thermo Scientific 10S spectrophotometer.

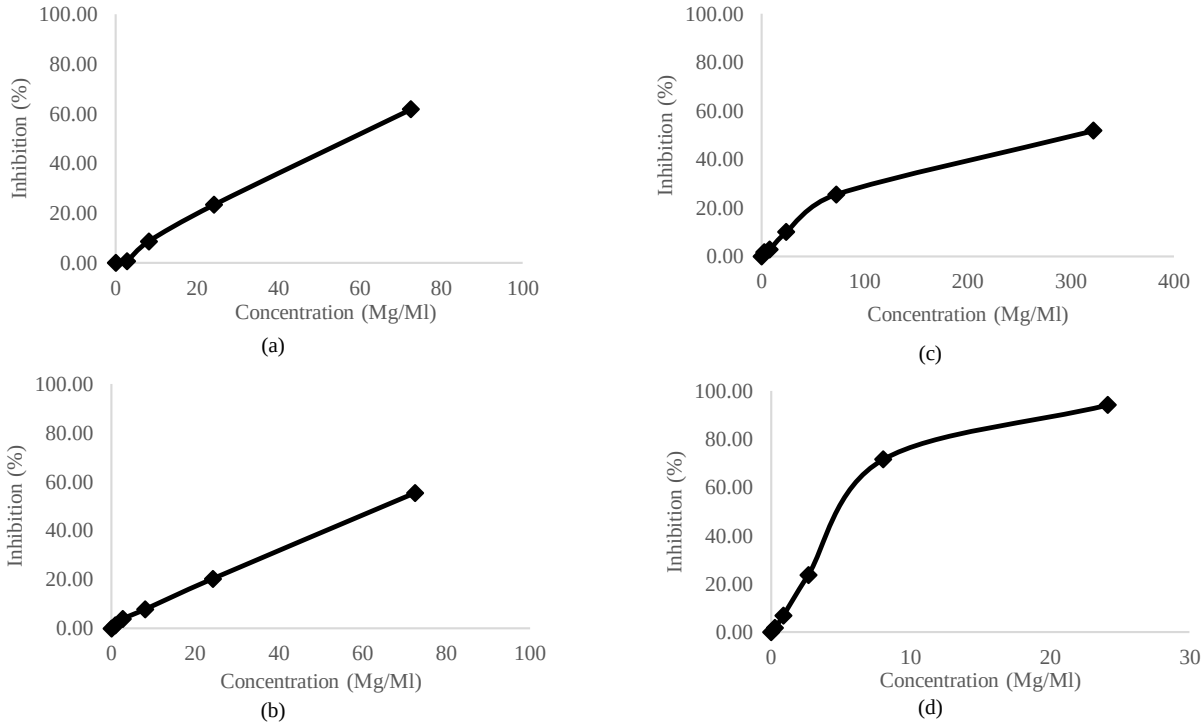

Figure. 2. Inhibition activity on DPPH radicals (a) Fraction C, (b) Fraction D, (c) Fraction E, (d) Gallic Acid.

C. Plant material

Leaves of C. odorata L. was collected in August 2017 from Ambon-Indonesia. The number of voucher specimen (48) was deposited in the Biology Laboratory, Faculty of Education and Teacher Training, Pattimura University, Ambon.

D. Extraction and Isolation

Dried leaves of *C. odorata* (2,76 kg) was extracted with methanol at room temperatur (3 x 24 hour). The solvent of methanol extract was removal through evaporation. The residue (270 g) was fractionated with *n*-hexane , CH_2Cl_2, EtOAc and MeOH by vacuum coloumn chromatography. As a result, 5 fraction (A-E) were obtained.

E. DPPH Radical Scavenging Assay

DPPH assay was used ny the method published previously [17]. Preparation of DPPH solution (6 x 10^{-5} M) by dissolving 2,37 mg of DPPH in 100 mL of methanol. Samples (Fraction A-E) 33 µL mix with 1 mL DPPH solution. The mixed sample solution was incubated for 20 minutes at room temperatur. The absorbance (As) of the mixture solution was measured by UV-Vis spectrophotometer at 517 nm. A standard is used gallic acid. The inhibitory activity was calculated by Equation (1). IC_{50} expressed as a quantity of an fraction inhibitory concentration against a half of DPPH radicals.

$$\text{Inhibition (\%)} = \left[\frac{(Ab-As}{Ab}\right] x\ 100 \qquad (1)$$

III. Results and Discussion

A. Fractination of methanol extract from C. odorata Leaves

Fractionation of the methanol extract of *C. odorata* was carried out gradually so that a single subfraction was obtained which showed a compound. The first stage, methanol extract of *C. odorata* (90 g x 3) was fractionated by vacuum chromatography column (VCC) using 60G silica gel (250 g x 3) and *n*-hexane (100%), CH_2Cl_2 (100%), EtOAc (100 %) and MeOH (100%) eluent gradient. The diameter and height of the columns used are 12 cm each. Fractionation results obtained 5 fractions (A-E), fractions A (6,8 g), B (27,83 g), C (43,43 g), D (29,56 g) and E (197,28 g).

B. Antioxidant Activity

Antioxidants are compounds that can protect the body from damage caused by free radicals. While free radicals are chemical compounds that have free electrons or unpaired electrons. Unpaired electrons are unstable so that they easily bind to other molecules and form unwanted reactions [15].

Antioxidant activity was carried out by DPPH (2,2-diphenyl-1-pikrilhidrazil) radical inhibition method. The A-E fraction from the methanol extract of *C. odorata* leaves was selected for in vitro antioxidant activity using DPPH method. This information is used to determine antioxidant activity in each fraction and potential compounds that have antioxidant activity in the fraction. The result of inhibitory activity from the A-E fraction of the methanol extract of *C. odorata* leaves against DPPH radicals is shown in Figure 1.

The inhibiting activity of samples of A-E fraction against DPPH radicals was carried out by reacting 1 mL of DPPH solution (6 x 10^{-5} M) with 33.3 µL samples in the microtube, then incubating for 20 minutes. The solution was measured for absorbance with a UV-Vis spectrophotometer at a wavelength of λ 517 nm. The positive control is gallic acid and methanol as blank. The reaction will cause discoloration of the solution from purple to yellow [16]. The inhibitory activity (%) is calculated by Equation (1).

The results shown in Figure 1 show that fraction D has a DPPH radical inhibitory activity better than other fractions (A, B, C and E). Furthermore the fractions C, D and E are determined by the IC_{50} value obtained from the regression curve equation (Figure 2). IC_{50} values from

each of the fractions C, D and E are 62.74; 67.72 and 110.29 µg / mL. The IC_{50} value of the gallic acid positive control was 14.89 µg / mL. IC_{50} results show that the fractions C and D have the potential for antioxidant activity which is better than the fractions A, B and E. Antioxidants will inhibit oxidation reactions by binding to free radicals thereby reducing the damage caused [15].

IV. CONCLUSION

The fractionation stage of the methanol extract of the *C. odorata* leaves obtained A-E fraction, showed inhibitory activity against DPPH radicals (2,2-difenill-1-pikrilhidrazil) on fractions C, D and E with IC_{50} values 63,95; 64.38 and 202.15 µg/mL. Positive control, which is gallic acid, has an IC_{50} value of 5.29 µg/mL.

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